

## Bacterial respiration of arsenic and selenium

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### Abstract

Oxyanions of arsenic and selenium can be used in microbial anaerobic respiration as terminal electron acceptors. The detection of arsenate and selenate respiring bacteria in numerous pristine and contaminated environments and their rapid appearance in enrichment culture suggest that they are widespread and metabolically active in nature. Although the bacterial species that have been isolated and characterized are still few in number, they are scattered throughout the bacterial domain and include Gram-positive bacteria, beta, gamma and epsilon *Proteobacteria* and the sole member of a deeply branching lineage of the bacteria, *Chrysiogenes arsenatus*. The oxidation of a number of organic substrates (i.e. acetate, lactate, pyruvate, glycerol, ethanol) or hydrogen can be coupled to the reduction of arsenate and selenate, but the actual donor used varies from species to species. Both periplasmic and membrane-associated arsenate and selenate reductases have been characterized. Although the number of subunits and molecular masses differs, they all contain molybdenum. The extent of the environmental impact on the transformation and mobilization of arsenic and selenium by microbial dissimilatory processes is only now being fully appreciated. © 1999 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Selenate reduction; Selenite reduction; Arsenate reduction

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## 1. Introduction

Arsenic and selenium are two elements whose potential toxic and teratogenic effects outweigh their relatively low crustal abundances. The extensive deaths and deformities in waterfowl and other wildlife attributed to micromolar concentrations of selenium at the Kesterton reservoir [1,2] and more recently, the outbreak of human arsenicosis in Bangladesh caused by contamination of drinking water wells with micromolar concentrations of arsenic are just two examples that underscore the need to understand the processes involved in the transformation and mobilization of these two elements. Field and laboratory studies conducted over the last decade and a half have provided evidence that reduction of arsenic and selenium oxyanions occurs primarily via microbial dissimilatory reduction. Several new species of bacteria that readily use either arsenate or selenate, or in some cases both, as terminal electron acceptors have only recently, however, been isolated and characterized. The purpose of this review is to highlight some of the recent advances in the taxonomy, physiology and biochemistry of arsenic and selenium respiring bacteria.

## 2. Environmental significance of arsenic and selenium

Arsenic and selenium are elements that naturally occur in aquatic and terrestrial environments. Arsenic is commonly found in weathered volcanic and marine sedimentary rocks, fossil fuels and a number of minerals (i.e. scorodite, arsenopyrite, realgar, orpiment) [3,4]. Selenium is found in fossil fuels, shales, alkaline soils and as a constituent in over 40 minerals (i.e. ferroselite, challomenite, schneiderite) [5–7]. Although the average crustal abundance of arsenic is less than 0.0002% [8] and of selenium less than 0.0001% [7], both elements can accumulate to micromolar concentrations under certain conditions. The erosion of Cretaceous marine sediments from the California Coastal Ranges is believed to have contributed to the enrichment of selenium in the San Joaquin Valley [9]. Anthropogenic activity, including drainage from mines and tailing wastes, the combus-

tion of coal and fuel oil (i.e. flue gas and fly ash) and runoff from irrigated seleniferous soils, also contributes to the accumulation of these two elements. They have been used in the formulation of pesticides, medicinals and semiconductors, as catalysts and pigments, and in glass manufacturing [10,11]. Toxic levels may occur as a result of a single event or the slow continual accumulation over prolonged periods of time. A major fish kill resulted from the discharge of selenium-rich fly ash from a lignite-fired power plant at Martin Lake (TX, USA) [12]. Selenium reached toxic levels in the Kesterton reservoir when selenium-rich subsurface water was mixed with surface irrigation runoff and repeatedly used to flood the reservoir [13].

Arsenate (As(V)) is the prevalent form of arsenic in oxic environments whereas arsenite (As(III)) is most common under anoxic conditions [14–17]. A transition from As(V) to As(III) through the oxycline has been documented for a number of stratified water bodies including Mono Lake (CA, USA) [14,15,18]. The behavior of arsenic species in sediments, however, is complicated by the binding of As(V) to hydrous manganese, aluminum and, especially, iron [4,18]. Hence, bacterial reduction of Fe(III) to Fe(II) can effect the arsenic solubility as the process should release any bound As(V), making it available for further chemical or biological reduction [19]. Such a mechanism has been proposed as the source of arsenic in the wells of Bangladesh. Arsenic-rich iron hydroxides in the alluvial aquifers of the Ganges delta that were initially deposited during the late pleistocene are now being reduced [20]. The reducing conditions may be the result of general microbial respiration or the specific activity of iron or arsenate respiring bacteria. Dissolution of As(III) in sediments from iron-arsenate oxides is readily enhanced by bacteria [21]. *Desulfotomaculum auripigmentum*, a recently described arsenate respirer, has been shown to grow with scorodite,  $((\text{FeAsO}_4) \cdot 2\text{H}_2\text{O})$ , as the source of arsenate [22,23]. Thus, it is possible that such microbes inhabit sediments in the Ganges aquifer and are growing on the naturally occurring arsenate-containing minerals. Alternatively, the release of arsenic may be due to the draw down of well water by human usage, resulting in the oxidation of arsenopyrite minerals that have come into contact with oxygen-rich water. This proc-

ess could also be contributing to the increase of dissolved arsenic species in the well water.

The transformation of arsenate occurs by microbial processes, however, its interaction with reduced ions (i.e. Fe(II),  $S^{2-}$ ) complicates assessing the relative contribution of each process in natural environments. Bacterial reduction of arsenate to arsenite has been detected in anoxic sediments [24,25]. Dowdle and colleagues [24] observed that As(V) reduction was inhibited by chloramphenicol, dinitrophenol, cyanide and tungstate. Sulfide, either abiotically or microbially produced, can also chemically reduce As(V) to As(III), resulting in the formation of minerals like orpiment ( $As_2S_3$ ). The occurrence of orpiment in sulfidic hot springs formations has been attributed to abiotic mechanisms [26]. *D. auripigmentum*, however, forms orpiment in culture by initially reducing As(V) and then sulfate during growth [22,23]. Therefore, the biological generation of orpiment and other reduced minerals of As(III) is also possible and their occurrence in nature cannot easily be attributed to either biotic or abiotic mechanisms.

The soluble oxyanions selenate (Se(VI)) and selenite (Se(IV)) are the primary forms of selenium in oxic environments. They disappear in the oxic/anoxic transition zone and are replaced by elemental selenium (Se(0)), the dominant species in anoxic sediments [27–29]. Se(VI) and Se(IV) reduction to insoluble Se(0) occurs in the presence of high levels of sulfate ( $> 300$  mM) such as was observed in evaporative brines in the San Joaquin Valley [30,31]. The transformation of selenium in nature occurs primarily by biotic processes [32]. Se(VI) does not readily undergo chemical reduction under physiological conditions of pH and temperature [30,33]. Thus, it is highly unlikely that abiotic reduction of selenate, such as by green rust [34], plays an important role in natural environments. In fact, biological selenate reduction has been shown to occur in chemically disparate environments [7,30,31,35–38]. The rate constants for these reactions occurring in situ can be quite rapid and reach values as high as  $\sim 2.0$   $h^{-1}$  [35,38]. Steinberg and Oremland [38] surveyed 11 different aquatic environments displaying a wide range of salinities (i.e. freshwater to extremely hypersaline) and pH (neutral to highly alkaline) and in only one case, an extremely saline and alkaline environment, was biological reduction not discernible.

The rates of selenate reduction followed Michaelis-Menten kinetics and the apparent  $K_m$  determined for six environments ranged from 7.9 to 720  $\mu M$  [38]. Assays of  $^{75}Se$ -selenate reduction to  $^{75}Se(0)$  did not show a lag period even in cases where selenate was not detectable in overlying waters or porewaters [38]. More recently, selenate reduction was detected in sediments from the Monongahela, Allegheny and Ohio rivers in Pittsburgh, PA, USA, [39] as well as Arthur Kill, New York, USA [40]. These findings suggest that bacteria capable of dissimilatory selenate reduction are abundant in nature and have a constitutive ability to reduce selenate.

### 3. Arsenic and selenium in biological systems

The impacts of arsenic and selenium on biological systems are concentration-dependent and vary from organism to organism. The need for selenium as a micronutrient has been well-established and there is evidence that suggests that arsenic may also be beneficial. Several different mechanisms provide resistance to elevated concentrations for a number of microorganisms and arsenate and selenate respiring bacteria actually require millimolar concentrations. We will confine our discussion here to arsenic and selenium as nutrients, mechanisms for their detoxification and their oxyanions as terminal electron acceptors (i.e. respiration).

#### 3.1. Nutrient requirement

Despite their toxicity, both arsenic and selenium may be actively sequestered in plant and animal tissue. Arsenobetaine is a major osmolyte in a number of sharks [41–43] and when degraded, it can be a source of inorganic arsenic [44]. Methylated arsenic compounds are also important in aquatic environments [45,46] and have been found in polysaccharides from marine algae [47]. Dimethylselenoniopropionate (DMSeP) is the Se-containing analog of the osmolyte dimethylsulfoniopropionate (DMSP) found in plants and algae. Both DMSP and DMSeP can be degraded to acrylate and dimethylselenide or dimethylsulfide by bacterial lyases [48]. DMSeP has been detected in algal populations found in Se-contaminated ponds [49]. Selenium is an essential ele-

ment for both prokaryotes and eukaryotes and is thus readily assimilated. Selenium deficiency has been associated with the decreased activity of selenium-containing enzymes such as glutathione peroxidase [50]. To counteract this deficiency, cow feed is often supplemented with selenium and as such represents another anthropogenic source of selenium. Selenium is found in selenocysteine-containing selenoproteins, selenium-containing tRNAs, as well as selenoenzymes [50,51]. The glycine reductase from *Clostridium sticklandii* and the formate dehydrogenases from *Methanococcus vannielii* and *Escherichia coli* are examples of selenoenzymes that contain selenocysteine [50]. The nicotinic acid hydroxylase from *C. barkeri* [52] and the xanthine dehydrogenase from *C. cylindrosporum* are selenium-dependent, but do not contain selenocysteine [50]. The mechanism of selenite assimilation into the cell is still unknown, but the reduction of selenite to selenide is believed to be via the thioredoxin-glutaredoxin system or alternatively via pathways involved in sulfur metabolism [50]. A greater discussion of selenoproteins and their biosynthesis may be found in the review by Heider and Boeck [50].

### 3.2. Toxicity

Exposure to a high concentration of arsenic and selenium is toxic to most microbes and death and birth defects in animals have been well-documented [12,13]. The toxicity of arsenic is due to its reversible combination with thiol groups and by substitution for phosphorus. Arsenic resistance, however, is widespread among clinical isolates of *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [53] and the mechanism of As(V) reduction by resistant microbes has been well-studied [54]. The genes for arsenate resistance can be plasmid borne or chromosomal [55–59]. Two plasmids, R773 from *E. coli* [57,58] and pI258 from *S. aureus* [59] have been well-characterized. The plasmid R773 contains four genes, *arsR*, *arsA*, *arsB* and *arsC* [58]. The plasmid pI258 contains three genes *arsR*, *arsB* and *arsC* [59]. In both cases, it is the *arsC* gene that encodes a soluble, 13-kDa polypeptide, that reduces As(V) to As(III). Arsenite is then exported out of the cell by either ATP-driven or chemiosmotic transport systems [60]. Diorio et al. [61] have also discovered

a functional chromosomal *ars* operon homolog. Southern analyses have revealed other chromosomal *ars* operon homologs in *Shigella sonnei*, *Citrobacter freundii*, *Enterobacter coloaecae*, *Salmonella arizonae*, *Erwinia carotovora*, *Klebsiella pneumoniae* and *P. aeruginosa*, suggesting that chromosomal arsenate resistance is widespread within the enterobacteriaceae [61].

The toxicity of selenium differs from that of arsenic in that it involves the substitution of selenium for sulfur in thiol-containing proteins. In animals, Se toxicity is conferred primarily by ingestion of food that is rich in seleniferous compounds (e.g. selenomethionine) as a consequence of chronic feeding/ingestion patterns of wildlife inhabitants of Se-contaminated food chains where bioconcentration and biological magnification effects occur. Selenium poisoning can result in conditions known as alkali disease and the blind staggers. Plants and microbes can incorporate inorganic selenium oxyanions into their tissues by assimilatory reduction to the level of selenide, as in selenocysteine, followed by their assembly into proteins. Several different mechanisms are known that detoxify selenium and involve either the reduction of Se(VI) and Se(IV) to Se(0) or Se(-II). A non-specific mechanism that results in resistance to elevated levels of selenate as well as arsenate and heavy metal oxyanions ( $\text{Cr}_2\text{O}_7^{2-}$ ,  $\text{Rh}_2\text{O}_3$ ,  $\text{Eu}_2\text{O}_5$ ,  $\text{TeO}_4^{2-}$ ,  $\text{TeO}_3^{2-}$ ) involves the regulation of intracellular redox poise [62,63]. Phototrophic prokaryotes like *Rhodobacter sphaeroides* build up reducing equivalents during photosynthesis. Redox poise is maintained through the removal of excess electrons via a membrane-bound,  $\text{FADH}_2$ -dependent metal reductase (MORase). *Wolinella succinogenes* [64], *Desulfovibrio desulfuricans* [65], specific strains of *Pseudomonas stutzeri* [66] and *E. coloaecae* [67] are capable of reducing sub-millimolar levels of Se(VI) to Se(0). This activity could be attributed to other reductases that have a broad range substrate specificity. The membrane-associated nitrate reductases of *E. coli* appear to be able to reduce Se(VI) as indicated by the oxidation of reduced methyl viologen [68]. The results of reductase activity assays using artificial electron donors such as benzyl and methyl viologen, however, should be viewed with caution. Membranes from selenate grown cells of *Sulfurospirillum barnesii* rapidly oxidize methyl viologen with tungstate or

Table 1

Comparison of free energies (in kcal mol<sup>-1</sup> e<sup>-</sup>) for various electron acceptors coupled to H<sub>2</sub> oxidation (modified with permission from Newman et al., [4])

Reaction	G' kcal mol <sup>-1</sup> e <sup>-</sup>
1/4 O <sub>2</sub> (g)+1/2 H <sub>2</sub> → 1/2 H <sub>2</sub> O	-23.55
1/2 MnO <sub>2</sub> (s)+H <sup>+</sup> +1/2 H <sub>2</sub> → 1/2 Mn <sup>2+</sup> +H <sub>2</sub> O	-22.48
1/5 NO <sub>3</sub> +1/5 H <sup>+</sup> +1/2 H <sub>2</sub> → 1/10 N <sub>2</sub> (g)+3/5 H <sub>2</sub> O	-20.66
1/2 SeO <sub>4</sub> <sup>2-</sup> +1/2 H <sup>+</sup> +1/2 H <sub>2</sub> → 1/2 HSeO <sub>3</sub> <sup>-</sup> +1/2 H <sub>2</sub> O	-15.53
1/8 NO <sub>3</sub> +1/4 H <sup>+</sup> +1/2 H <sub>2</sub> → 1/8 NH <sub>4</sub> <sup>+</sup> +3/8 H <sub>2</sub> O	-13.42
1/3 CrO <sub>4</sub> <sup>2-</sup> +5/3 H <sup>+</sup> +1/2 H <sub>2</sub> → 1/3 Cr <sup>3+</sup> +3 H <sub>2</sub> O	-10.76
Fe(OH) <sub>3</sub> (am)+2 H <sup>+</sup> +1/2 H <sub>2</sub> → Fe <sup>2+</sup> +3 H <sub>2</sub> O	-10.4
1/4 HSeO <sub>3</sub> <sup>-</sup> +2 H <sup>+</sup> +1/2 H <sub>2</sub> → 1/4 Se <sup>0</sup> +3/4 H <sub>2</sub> O	-8.93
1/2 H <sub>2</sub> AsO <sub>4</sub> <sup>-</sup> +1/2 H <sub>2</sub> → 1/3 As <sup>0</sup> +1/2 H <sub>2</sub> O	-5.51
1/3 H <sub>3</sub> AsO <sub>3</sub> +1/2 H <sub>2</sub> → 1/3 As <sup>0</sup> +H <sub>2</sub> O	-2.58
1/8 SO <sub>4</sub> <sup>2-</sup> +1/8 H <sup>+</sup> +1/2 H <sub>2</sub> → 1/8 HS <sup>-</sup> +1/2 H <sub>2</sub> O	-0.10

phosphate, yet, neither can be used as terminal electron acceptors [69]. *D. desulfuricans* and two species of phototrophic bacteria also reduce sub-millimolar levels of Se(VI) to Se(-II) [70,71]. *Rhodocyclus tenuis* and *Rhodospirillum rubrum* produce both dimethylselenide and dimethyldiselenide from selenate while growing phototrophically [71]. *R. tenuis* also produces dimethylselenide from selenite [71]. The methylation of organometalloids has been presented in greater depth in the review by Gadd [72].

### 3.3. Respiration

Although the reduction of oxyanions of arsenic and selenium can occur by a number of different mechanisms, the most environmentally significant process is dissimilatory reduction. Both arsenate and selenate are electrochemically positive with oxidation-reduction potentials of +139 mV and +425 mV, respectively [73]. A comparison of the calculated free energies for a number of electron acceptors us-

ing hydrogen as the electron donor reveals that the reduction of Se(VI) to Se(IV) falls between manganese reduction and nitrate reduction to ammonium ([4], Table 1). The reduction of As(V) to As(III) falls between Se(IV) reduction to Se(0) and sulfate reduction (Table 1). This comparison shows that selenate and arsenate could be environmentally significant electron acceptors. Clearly, this would be dependent on the concentrations of the specific oxyanions and the availability of suitable electron donors. However, it does suggest that selenate reduction should occur before nitrate reduction to ammonium and arsenate reduction should occur before sulfate reduction. Thermodynamic calculations using acetate or lactate as the electron donor suggest that both arsenate and selenate reduction are energetically favorable. Using the stoichiometry for the reactions listed in Table 2, the reduction of As(V) to As(III) theoretically yields -172 kJ mol<sup>-1</sup> of lactate [74] while the reduction of Se(VI) to Se(IV) yields -575 kJ mol<sup>-1</sup> of acetate and -343 kJ mol<sup>-1</sup> of lactate [75,76]. Given the toxic nature of both these elements, however, it was not until recently, with the isolation and characterization of several species of arsenate and selenate reducing bacteria, that this type of respiration was confirmed.

### 4. Diversity of arsenic and selenium respiring prokaryotes

Microbes that respire oxyanions of arsenic and selenium are not confined to any particular group of prokaryotes (Table 3) and are found throughout the bacteria domain (Fig. 1). Their physiological characteristics vary greatly, from species with very limited metabolic capabilities to others that are quite versatile. In fact, the ability to respire arsenate or

Table 2

Stoichiometry for the reduction of oxyanions of arsenic and selenium

Reaction	Organisms	References
Acetate <sup>-</sup> +2 HAsO <sub>4</sub> <sup>2-</sup> +2 H <sub>2</sub> AsO <sub>4</sub> <sup>-</sup> +5 H <sup>+</sup> → 4 H <sub>3</sub> AsO <sub>3</sub> +2 HCO <sub>3</sub> <sup>-</sup>	<i>C. arsenatis</i>	[93]
Acetate <sup>-</sup> +4 SeO <sub>4</sub> <sup>2-</sup> +H <sup>+</sup> → 2 CO <sub>2</sub> +4 SeO <sub>3</sub> <sup>2-</sup> +2 H <sub>2</sub> O	<i>T. selenatis</i>	[75]
Lactate <sup>-</sup> +2 HAsO <sub>4</sub> <sup>2-</sup> +2 H <sup>+</sup> → 2 H <sub>2</sub> AsO <sub>3</sub> <sup>-</sup> +acetate <sup>-</sup> +HCO <sub>3</sub> <sup>-</sup>	<i>S. barnesii</i> , <i>D. auripigmentum</i> , <i>B. selenitireducens</i> , <i>B. arsenicoselenatis</i>	[76,78]
Lactate <sup>-</sup> +2 SeO <sub>4</sub> <sup>2-</sup> → acetate <sup>-</sup> +2 SeO <sub>3</sub> <sup>2-</sup> +HCO <sub>3</sub> <sup>-</sup> +H <sup>+</sup>	<i>S. barnesii</i> , <i>B. arsenicoselenatis</i>	[76,78]
Lactate <sup>-</sup> +SeO <sub>3</sub> <sup>2-</sup> +H <sup>+</sup> → Se(0)+acetate <sup>-</sup> +HCO <sub>3</sub> <sup>-</sup> +H <sub>2</sub> O	<i>B. selenitireducens</i>	[76,78]
3 Lactate <sup>-</sup> +2 SeO <sub>4</sub> <sup>2-</sup> +H <sup>+</sup> → 2 Se(0)+3 acetate <sup>-</sup> +3 HCO <sub>3</sub> <sup>-</sup> +2 H <sub>2</sub> O	<i>B. arsenicoselenatis</i> + <i>B. selenitireducens</i>	[76,78]

selenate (or both) is a characteristic that can be useful in distinguishing closely related species.

To date, three species of selenate respiring bacteria have been well-characterized, *Thauera selenatis*, *S. barnesii* and *Bacillus arsenicoselenatis* [75–78]. A fourth organism, *Bacillus selenitireducens*, is able to respire selenite [78]. All four species can reduce selenium oxyanions to Se(0) and the accumulations of this element are exogenous, occurring outside of the cell envelope in the surrounding growth milieu rather than as internalized precipitates or structures. A large number (~25) of newly isolated strains of *Aeromonas hydrophila* were able to reduce millimolar levels of Se(VI) and growth of the type strain (ATCC 7966) on Se(VI) with glycerol as the electron donor has been demonstrated [79]. There is a preliminary report of a strain of *W. succinogenes* isolated from the rumen that grows on Se(VI) [80]. In addition, several strains that can achieve growth by dissimilatory reduction of Se(VI) still await complete characterization. They include a very halophilic Gram-negative rod isolated from the Dead Sea, a coccus from the termite hindgut [81] and two strains (AK4OH1, Ke4OH1) belonging to the gamma subgroup of the

*Proteobacteria* that grow on aromatic compounds [82]. It is also likely that some novel species from the Euryarchaeota (i.e. extreme halophiles) and Crenarchaeota (i.e. thermoacidophiles) exist that can respire selenate, but as yet, their isolations have not been reported.

*T. selenatis*, is a Gram-negative, motile rod that was isolated from sediments of the San Joaquin Valley using mineral media with acetate as the electron donor [75]. Originally described as a pseudomonad, it is a facultative aerobe belonging to the beta subclass of the *Proteobacteria* [77] (Fig. 1). *T. selenatis* can also respire nitrate and the reported molar growth yields ( $Y_M$ ) are 57 and 55 g dry weight  $\text{mol}^{-1}$  acetate for selenate and nitrate, respectively [83]. Initial characterization of selenate and nitrate reduction has shown that they are separate pathways with distinct terminal reductases [84,85]. Nitrate respiration by *T. selenatis* has been reported as linked to denitrification based primarily on the detection of  $\text{N}_2\text{O}$  in the headspace and the absence of any accumulation of ammonium [77]. A complete stoichiometric balance of the reactants and products of nitrate respiration, however, has not been published.

Table 3  
Diversity of selenium and arsenic respiring bacteria

Species	Environment	Taxonomic affiliation	Electron donors	Electron acceptors
<i>C. arsenatis</i>	Gold mine waste water	Bacteria	Acetate	As(V), $\text{NO}_3^-$
<i>D. auripigmentum</i>	Upper Mystic Lake, MA, USA	Low G+C Gram-positive	$\text{H}_2$ , $\text{H}_2$ +acetate, lactate, pyruvate, butyrate, malate, ethanol, glycerol	As(V), $\text{SO}_4^{2-}$ , $\text{SO}_3^{2-}$ , $\text{S}_2\text{O}_3^{2-}$ , fumarate
<i>B. selenitireducens</i>	Mono Lake, CA, USA	Low G+C Gram-positive	Lactate, pyruvate	Se(IV), As(V), fumarate, $\text{NO}_3^-$ , $\text{NO}_2^-$ , trimethylamine oxide, low $\text{O}_2$
<i>B. arsenicoselenatis</i>	Mono Lake, CA, USA	Low G+C Gram-positive	Lactate, malate	Se(VI), As(V), fumarate, Fe(III), $\text{NO}_3^-$
<i>S. arsenophilum</i>	Aberjona watershed, MA, USA	Epsilon <i>Proteobacteria</i>	Lactate, pyruvate, $\text{H}_2$ +acetate	As(V), $\text{NO}_3^-$ , $\text{NO}_2^-$ , low $\text{O}_2$
<i>S. barnesii</i>	Massie Slough, NV, USA	Epsilon <i>Proteobacteria</i>	Lactate, pyruvate, $\text{H}_2$ +acetate	Se(VI), $\text{NO}_3^-$ , $\text{NO}_2^-$ , As(V), Fe(III), fumarate, S(0), $\text{S}_2\text{O}_3^{2-}$ , low $\text{O}_2$
<i>Aeromonas hydrophila</i> ATCC 7966	'...Tin of milk with a fishy odor'	Gamma <i>Proteobacteria</i>	Glycerol	Se(VI), Fe(III), $\text{NO}_3^-$ , Co(III), fumarate
<i>T. selenatis</i>	San Joaquin Valley, CA, USA	Beta <i>Proteobacteria</i>	Acetate, glucose	Se(VI), $\text{NO}_3^-$ , $\text{O}_2$
AK4OH1	Arthur Kill, NY, USA	Gamma <i>Proteobacteria</i>	4-Hydroxybenzoate	Se(VI)
Ke4OH1	Kesterton reservoir, CA, USA	Gamma <i>Proteobacteria</i>	4-Hydroxybenzoate	Se(VI)

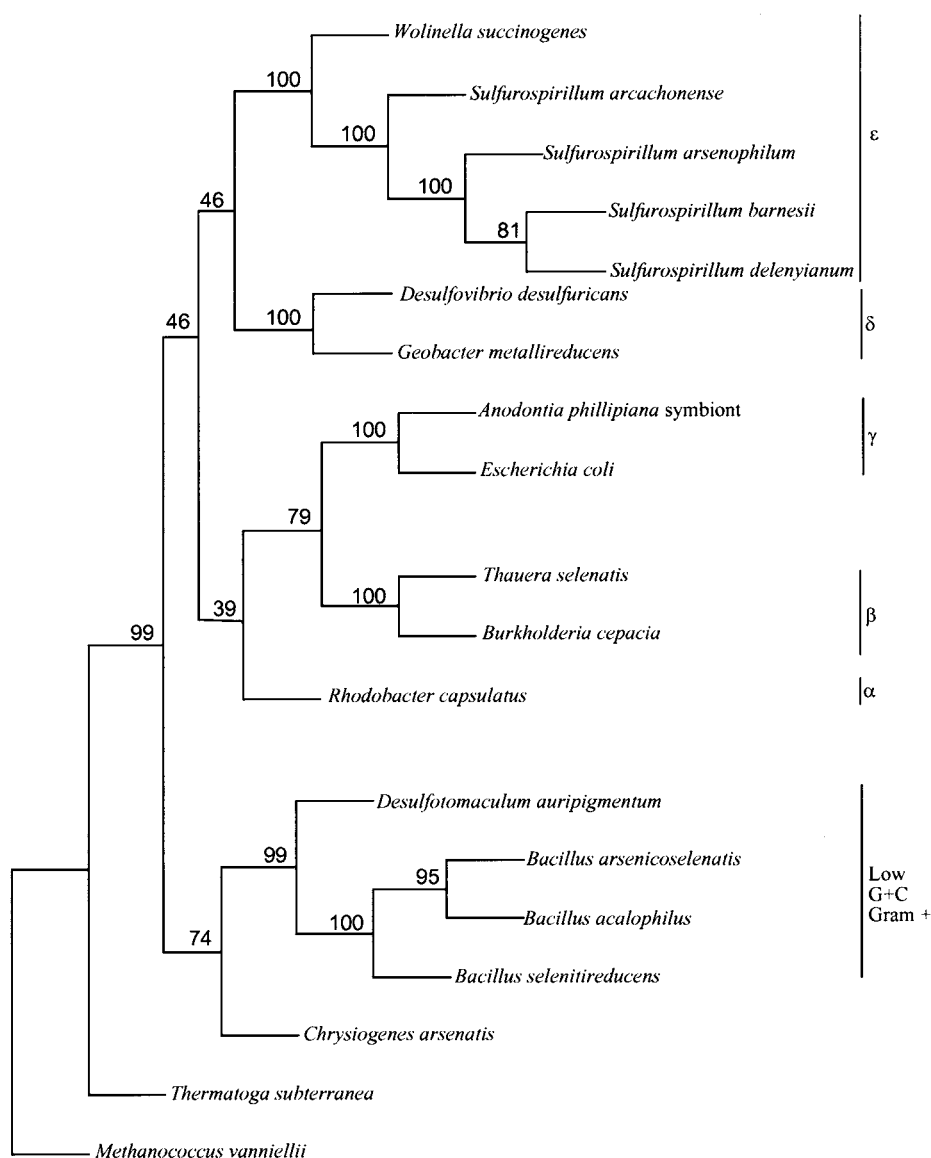


Fig. 1. Phylogenetic tree based on 16S rDNA sequences of bacteria that respire oxyanions of selenate and arsenate. The maximum parsimony tree was constructed using PHYLIP [97]. A total of 1200 bases were used and bootstraps (based on 100 replicates) are given at the nodes. Both AK4OH1 and KeOH1 branch with the gill symbiont of *Anodontia phillipiana* [82]. The branch position of *C. arsenatis* with the low G+C Gram-positives branch probably occurred because the tree does not include representatives of the cyanobacteria, Chloroflexaceae or flavobacteria.

Therefore, it would be of great interest to determine if  $N_2O$  is the terminal endproduct because it lacks a terminal  $N_2O$  reductase.

*S. barnesii* strain SES-3 is a motile, vibrioid-shaped, Gram-negative bacterium that was isolated

from an enrichment culture with acetate as the electron donor [86]. Once isolated, however, it was shown to grow with lactate or pyruvate as electron donors.  $H_2$  could also be used as an electron donor when acetate was provided as the carbon source.

Small quantities of yeast extract are routinely employed as a nutritional supplement in its culture medium to improve yields. However, the organism will not grow with yeast extract as the electron donor. *S. barnesii* can achieve respiratory growth with a variety of electron acceptors including arsenate, nitrate, thiosulfate, elemental sulfur, ferric iron, manganic ion and fumarate in addition to selenate and is also capable of weak microaerobic growth [74,76]. Of great significance is that it does not use sulfate as an electron acceptor. It was initially believed, because sulfur and selenium are closely related group VIB elements, that selenate reduction could be attributed to sulfate reducing bacteria [70]. The characterization of *S. barnesii* and other selenate respirers has clearly shown that the dissimilatory reduction of sulfate to sulfide and Se(VI) to Se(0) are achieved by very different microbes using different biochemical pathways and with much different cellular energy yields. Growth of *S. barnesii* on nitrate is by dissimilatory reduction to ammonium and a stoichiometric balance is achieved between nitrate and ammonium in cell suspensions. Although traces of  $N_2O$  accumulate in the headspace during incubation with nitrate, there is no enhanced accumulation in the presence of acetylene, which blocks  $N_2O$  reductase. The  $Y_M$  values for growth on selenate and nitrate are 11.5 and 7.1 g mol<sup>-1</sup> lactate, respectively [74,76]. *S. barnesii* has only recently been assigned to the genus *Sulfurospirillum* of the epsilon subgroup of the *Proteobacteria* [87]. Several publications describing its ability to grow on iron [88,89] and preliminary biochemical characterization [90] used the proposed name *Geospirillum barnesii*. Phylogenetic analysis based on the 16S rRNA sequence suggested that *S. barnesii* was closely related to the type species *Sulfurospirillum deleyianum* and the newly described marine organism *S. arcachonense* [91]. Indeed, *S. barnesii*, *S. deleyianum* and the arsenate respiring bacterium *S. arsenophilum* share a 97% sequence similarity [87]. In fact, all known *Sulfurospirillum* species form a distinct clade in the epsilon *Proteobacteria* (Fig. 1). DNA/DNA hybridization, however, has shown that *S. deleyianum*, *S. barnesii* and *S. arsenophilum* are indeed distinct species [87]. Furthermore, of the three species, *S. barnesii* is the only one capable of dissimilatory selenate reduction.

*B. arsenicoselenatis* and *B. selenitireducens* are

novel species of Gram-positive bacteria that respire selenium and arsenic oxyanions. Originally designated E-1H and MLS10, respectively, they were isolated from Mono Lake, CA, USA, an alkaline (pH=9.8) and hypersaline (salinity=90 g l<sup>-1</sup>) soda lake [78]. Both isolates are moderately halophilic, as well as alkaliphilic, exhibiting maximal growth at a pH range between 9 and 11 and salinities between 40 and 120 g l<sup>-1</sup> [78]. Strain E-1H is a spore former and strict anaerobe that grows by reducing Se(VI) to Se(IV). It can use lactate or malate as electron donors as well as starch (Table 3). Strain MLS-10 does not form spores and grows by reduction of Se(IV) to Se(0). Although a number of microbes are capable of reducing highly toxic Se(IV) to Se(0) for the purpose of detoxification, strain MLS-10 is the first organism that can respire this substrate to sustain growth and achieves this at elevated Se(IV) concentrations (i.e. 10 mM). When the two strains are grown in co-culture, Se(VI) is quantitatively reduced to elemental selenium (Table 2). MLS-10 can use lactate or pyruvate as electron donors, grows fermentatively on glucose, fructose or starch and is also capable of microaerobic growth. Both strains can also respire arsenate. Phylogenetic analysis places both *B. arsenicoselenatis* and *B. selenitireducens* within group 6 of the low G+C Gram-positives along with other alkaliphilic species including *B. alkalophilus* [78] (Fig. 1). Enrichment cultures from the three rivers of Pittsburgh, PA, USA, suggest that there are freshwater neutrophilic species of selenate respiring spore-forming bacilli as well [39].

There are six species of organisms known to grow by dissimilatory arsenate reduction (Table 3). They are also not confined to any particular group of bacteria and are distributed throughout the bacteria domain (Fig. 1). Among these are several species that have been described above that also respire oxyanions of selenium. *S. barnesii* reduces As(V) to As(III) with a  $Y_M$  for growth on As(V) of 5.3 g mol<sup>-1</sup> lactate [74]. *B. selenitireducens* and *B. arsenicoselenatis* also respire As(V) to As(III) [78].

Three other species are capable of growth on As(V). These include strain MIT-13 [92], now classified as *Sulfurospirillum arsenophilum* [87], *Desulfotomaculum auripigmentum* [23] and *Chrysiogenes arsenatis* [93]. *S. arsenophilum* is a Gram-negative, vibrioid-shaped, microaerobic sulfur reducing bacte-

rium that was isolated from arsenic-contaminated watershed sediments in eastern Massachusetts [92]. It is closely related to *S. barnesii* and other members of the *Sulfurospirillum* clade in the epsilon *Proteobacteria* [87]. It grows by coupling arsenate reduction to the complete oxidation of lactate to CO<sub>2</sub>. *S. arsenophilum* can also use pyruvate as an electron donor as well as hydrogen if acetate is provided as a carbon source [87].

*D. auripigmentum* strain OREX-4 is a low G+C Gram-positive, that was isolated from surface lake sediments also in eastern Massachusetts [22,23]. The reported  $Y_M$  for growth on As(V), 5.6 g mol<sup>-1</sup> lactate [23], is similar in value to that of *S. barnesii*. It is also able to use sulfate as a terminal electron acceptor [22]. It is interesting to note that the growth yield on sulfate (2.3 g mol<sup>-1</sup> lactate) is less than that for arsenate and in agreement with the higher free energy for arsenate reduction [23]. When grown in the presence of both arsenate and sulfate, it produces the arsenic sulfide mineral orpiment both intra- and extracellular [22,23]. Although *D. auripigmentum* is a low G+C Gram-positive, it is not closely related to either *B. arsenicoselenatis* or *B. selenitireducens*, but does group with other *Desulfotomaculum* species (Fig. 1).

*C. arsenatis* strain BAL-1T, a strict anaerobe isolated from gold mine wastewater at Ballarat Goldfields in Australia [93], represents its own deeply branching lineage in the bacteria domain. That it branches off the low G+C Gram-positive branch in our maximum parsimony tree is probably the result of that for the sake of clarity, we did not include representatives of the cyanobacteria, Chloroflexaceae, or flavobacteria (Fig. 1). *C. arsenatis* couples the oxidation of acetate to the reduction of arsenate (Table 2). It cannot use Fe(III), sulfate or thiosulfate as terminal electron acceptors, but does respire nitrate [93].

## 5. The biochemistry of arsenate and selenate reduction

Dissimilatory arsenate and selenate reduction is carried out by distinct terminal reductases that are typically associated with a cytochrome. To date, only the arsenate reductase from *C. arsenatis* and the selenate reductase from *T. selenatis* have been char-

acterized to any detail. Characterization of the arsenate and selenate reductases from *S. barnesii* is currently ongoing.

The arsenate reductase from *C. arsenatis* is a heterodimer, consisting of a major subunit of 87 kDa and a minor subunit of 29 kDa with a native molecular mass of 123 kDa [94]. It contains molybdenum, iron, acid-labile sulfur and zinc. The apparent  $K_m$  of the enzyme has been reported as 300  $\mu$ M with a  $V_{max}$  of 7 mmol arsenate reduced per min per mg of protein [94]. The enzyme is substrate specific as nitrate, sulfate, selenate or fumarate did not oxidize benzyl viologen.

Unlike *C. arsenatis*, the arsenate reductase from *S. barnesii* is membrane-bound. An enzyme complex that exhibits arsenate reductase activity has been purified by preparative gel electrophoresis. A trimeric complex, with a calculated molecular mass of about 100 kDa, has an  $\alpha$  subunit of 65 kDa, a  $\beta$  subunit of 31 kDa and a  $\gamma$  subunit of 22 kDa [4]. A *b*-type cytochrome with a difference spectrum with absorbance maxima at 557, 520 and 416 nm has also been detected in the membrane fraction. Although a complete metals analysis has yet to be done, evidence for Fe-S prosthetic groups has been found. Furthermore, tungstate inhibits 80% of the arsenate reductase activity, strongly suggesting that molybdenum is also present [95]. The enzyme is able to couple the reduction of As(V) to As(III) to the oxidation of methyl viologen and has an apparent  $K_m$  of 200  $\mu$ M [4].

The dissimilatory selenate reductase of *T. selenatis* is a periplasmic trimeric enzyme [85]. The complex has an apparent molecular mass of 180 kDa with an  $\alpha$  subunit of 96 kDa, a  $\beta$  subunit of 40 kDa and a  $\gamma$  subunit of 23 kDa [85]. The reductase contains molybdenum, iron and acid-labile sulfur and has an apparent  $K_m$  of 16  $\mu$ M. One of the subunits, presumably the 23-kDa protein, is a cytochrome *b* with a difference spectrum with absorbance maxima at 558, 528 and 424 nm [85]. The enzyme is very substrate specific, reducing only selenate to selenite. Nitrate, nitrite, chlorate or sulfate were not oxidized by the enzyme when benzyl viologen was used as the artificial electron donor [85].

Selenate reduction in *S. barnesii* is apparently very different than that in *T. selenatis*. Selenate reductase activity has been localized in the membrane fraction

[90]. Although a *b*-type cytochrome has been detected in the membrane fraction from selenate grown cells, its difference spectrum is different than that detected in *T. selenatis*, with absorbance maxima at 554, 523 and 422 nm [90]. Membrane fractions from cells grown on selenate exhibited the greatest activity (measured as  $\mu\text{g}$  methyl viologen oxidized per min per mg of protein) with selenate, but also had an appreciable activity with nitrate, thiosulfate and fumarate, even though components of these reductive pathways were not readily detectable in sodium dodecyl sulfate-polyacrylamide gel electrophoresis [90]. This suggests that the substrate specificity of the selenate reductase is apparently much broader than that of the enzyme from *T. selenatis*. Alternatively, it is possible that low levels of several terminal reductases (i.e. nitrate reductase) are constitutively expressed. Distinct selenate, fumarate, nitrate and arsenate reductases have been identified. The enzyme complex purified from membranes of selenate grown cells that exhibited selenate reductase activity is a heterotetramer with subunits of 82, 53, 34 and 21 kDa and has an apparent  $K_m$  of 12  $\mu\text{M}$  [69]. Dialysis with tungstate was shown to inhibit over 80% of the selenate reductase activity, suggesting that the enzyme contains molybdenum in the active site [95], although metal analysis has yet to confirm this.

That both dissimilatory selenate and arsenate reduction involve enzymes with molybdenum co-factors is significant. Mononuclear molybdenum enzymes have diverse functions and have been grouped into three major families: (1) the xanthine oxidase family, (2) the sulfite oxidase family and (3) the dimethylsulfoxide (DMSO) reductase family [96]. Periplasmic and membrane-bound dissimilatory nitrate reductases and trimethylamineoxide reductases are members of the DMSO reductase family. They are grouped together because they share a significant amino acid sequence similarity. The preliminary N-terminus amino acid sequence of the arsenate reductase of *C. arsenatis* revealed a high degree of sequence similarity with the polysulfide reductase of *W. succinogenes* and formate dehydrogenase of *E. coli* [94]. This suggests that it also belongs to the DMSO reductase family. The affiliations of the selenate reductase from *T. selenatis* and the arsenate and selenate reductases from *S. barnesii* remain to be established. Regardless, these new enzymes provide

another glimpse at how bacteria have utilized molybdenum as a co-factor for oxidoreductive reactions.

## 6. Conclusions

The otherwise toxic oxyanions of arsenate and selenate are suitable terminal electron acceptors for selected bacteria, providing enough energy for active growth and metabolism. Their reduction can be coupled to the oxidation of a variety of organic substrates including acetate, lactate and aromatics. The terminal reductases contain molybdenum and may be either located in the periplasmic or membrane-associated. Arsenate and selenate respiring bacteria have been shown to occur in a wide range of environments and not confined to any specific genus. The full impact of their activities on the biogeochemical cycling of arsenic and selenium is only now being realized. Furthermore, these organisms, and perhaps the enzymes themselves, may be useful in the bioremediation of selenium- and arsenic-contaminated environments.

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